Spider sedation induced by defensive chemicals of milliped prey*

(predator–prey interaction/co-evolution/quinazolinones/glomerin/homoglomerin)

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ABSTRACT   Wolf spiders (*Lycosa* spp.) show delayed induced sedation (total immobilization) of prolonged duration (in the order of days) after attacks upon millipeds (*Glomeris marginata*). The sedation is specifically attributable to glomerin and homoglomerin, two previously characterized quinazolinones present in the defensive secretion of *Glomeris*. Median sedative doses for the quinazolinones are in the range of 1–7 μg per spider, a fraction of the total (60–90 μg) present in the secretion of medium to full-grown millipeds. A sedative effect upon an invertebrate predator has not previously been demonstrated for an animal defense. Quinazolinones include the synthetic drug methaqualone (Quaalude), a potent human sedative.

Two of the more intriguing compounds recently isolated from arthropods are glomerin and homoglomerin, products of the defensive glands of *Glomeris marginata*, an oniscomorph milliped (1–4). The compounds are quinazolinones, closely related structurally to arborine, a plant natural product, and to methaqualone (Quaalude), a synthetic drug. Both arborine and methaqualone are sedative to vertebrates, the latter compound being widely used for that purpose as a human drug (5, 6). We here present evidence that glomerin and homoglomerin are potent sedatives to lycosid spiders, important potential enemies of millipeds, providing a demonstration of sedation as a mode of action for a defensive chemical upon an invertebrate predator.
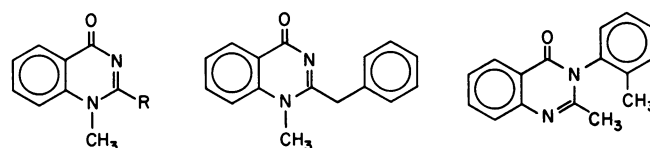
Glomerin (R=CH₃)
Homoglomerin (R=C₂H₅)   Arborine   Methaqualone

Glomeris marginata is native to Western Europe, where it is locally abundant, mostly in leaf litter (7). It responds to slight disturbance by coiling and to more persistent disturbance by emitting droplets of sticky clear fluid from the eight middorsal openings of its defensive glands (Fig. 1 *A* and *B*). Glomerin and homoglomerin are dissolved in this fluid, which derives its stickiness from uncharacterized proteinaceous components (4).

Our first clue to the sedative action of the secretion stemmed from observation of staged attacks of lycosid spiders upon *G. marginata*, which sometimes resulted in the spiders becoming immobilized for lengthy periods after exposure to the millipeds' secretion. We here describe these encounters, present quantitative data on the quinazolinone output of *G. marginata*, and show that these compounds, at

their natural concentrations in milliped secretion, can induce immobilization (= sedation) in lycosid spiders.

MATERIALS AND METHODS

The *G. marginata* stemmed from two localities (Exeter, England; Wageningen, Holland) and comprised specimens of all sizes and both sexes (body mass up to >60 mg). They were maintained in terraria and fed decaying leaves. When touched, they readily gave off secretion, but they could be moved about with a cold spoon, or by coaxing with a brush, without being caused to discharge.

The lycosid spiders were collected near Lake Placid, FL, at the Archbold Biological Station, and were maintained individually on moist sand in small plastic enclosures, which also served as arenas for the experimental feeding tests. For routine sustenance they were given a mealworm (larva of *Tenebrio molitor*) twice weekly; prior to experimentation they were starved 5–7 days. They were of relatively uniform size ($\bar{x} \pm$ SEM; body mass = 350 ± 10 mg; n = 1,290). Most were *Lycosa ceratiola* (identification of 146 spiders of our sample showed: 90% *L. ceratiola*; 5% *L. timuqua*; 3% *L. osceola*; <1% of each *L. miami*, *L. hentzi*, and *L. angusta*).

In all tests, individual millipeds and spiders were used only once. Synthetic glomerin and homoglomerin (collectively referred to hereafter as quinazolinones) were used throughout, except where otherwise indicated. Synthesis was by pathways previously described (1).

Spider–Milliped Encounters. These tests (n = 89) involved presenting single millipeds (medium to full sized) to individual spiders and recording the following events: duration of the attack (from moment spider contacted milliped to moment of disengagement of the pair), whether or not the milliped was bitten (whether it was seized in the spider's chelicers or merely pounced upon), whether the milliped was injured and had visibly given off secretion, and whether or not the spider became sedated after the encounter. The criterion for sedation—in these tests as in all others—was the spider's inability to right itself promptly when flipped on its back with a curved glass rod. A "normal" spider either regained its upright stance immediately or (more often) successfully resisted being turned on its back. Spiders were checked for sedation at 12 and 24 hr after the encounters and at daily intervals thereafter.

Secretory Output of Milliped. Output was measured by grasping individual preweighed millipeds (n = 8) in forceps after they had coiled, squeezing them gently so as to induce them to give off secretion (as in Fig. 1*B*), and taking up the discharged fluid in volumetrically calibrated tubes for subsequent analysis of quinazolinone content. The analytical technique was as described (1).

Antifeedant Potency of Quinazolinones. These tests (n = 240, including 30 controls), intended to determine whether the spiders are orally sensitive to quinazolinones and able to

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discriminate against the compounds on the basis of taste, involved offering individual mealworms to single spiders and delivering a droplet (5 μl) of quinazolinone solution to the spider's chelicers as soon as the mealworm had been firmly grasped by the spider and had ceased struggling. Dosages of quinazolinone that led to rejection of the mealworm within 3 min of application of the droplet were scored as antifeedant (consumption of a mealworm by a spider ordinarily takes >2 hr). Droplet delivery was effected with a micrometer-operated microsyringe. Egg albumin was used as a solvent for the quinazolinones and was itself tested as the control. Glomerin and homoglomerin, as well as an equimolar mixture of the two compounds, were each tested at seven dosages (*n* = 10 spiders per dosage) in the range of 1–150 μg per droplet.

Sedative Potency of Quinazolinones: *per os.* These tests (*n* = 418, including 68 controls) were intended to determine the systemic sensitivity of spiders to quinazolinones taken per mouth. Protocol was as in the antifeedant tests (same test samples, droplet size, dosage application procedure, and quinazolinone dosage range) except that the spiders were checked for induced sedation 12 and 24 hr after testing (and for recovery at daily intervals thereafter), and the samples contained an added nuclide label (^{125}I, ^{22}Na) that permitted subsequent calculation (from determination of spider body radioactivity) of the amount of quinazolinone ingested by the individual spiders (*n* = 15–20 spiders per dosage). Nuclide/albumin solution served as control. The data included a set of values obtained with a sample of freshly "milked" *Glomeris* secretion (50 millipeds), pre-analyzed for quinazolinone content and molar ratio, and diluted with nuclide/albumin solution to provide a series of graded quinazolinone concentrations matching that of the synthetic compounds.

Sedative Potency of Quinazolinones: Injected. These tests, (*n* = 543, including 85 controls), essentially a parallel to the preceding, measured sensitivity of the spiders to quinazolinone administered by injection. Injection (5 μl) was effected with a micrometer-operated microsyringe, into the "abdomen" (technically, the opisthosoma) of the spider. Glomerin, homoglomerin, and an equimolar mixture of the two compounds were injected at six dosages (*n* = 20–57 spiders per dosage) in the range of 1–25 μg per spider. Spider saline (8) was used as sample solvent and was itself tested as the control. Spiders were checked daily for sedation; a record was kept of which spiders died without ever having recovered from the induced sedation.

Data Presentation. Data from the antifeedant and sedative potency tests were summarized by probit calculation of median effective doses (ED$_{50}$s) and are presented as $\bar{x}$ ± SEM and (calculated) dynamic range (ED$_{05}$–ED$_{95}$) (9). The difference between any two ED$_{50}$s was tested by using Student's *t* distribution. Values of *t* were calculated by using the equation, derived from Finney (9),

$$t = \frac{m_1 - m_2}{\sqrt{SE_{m_1}^2 + SE_{m_2}^2}},$$

in which $m_i = \log ED_{50_i}$, SE_{m_i} = standard error of m_i, and *df* = $n_1 + n_2 - 4$.

RESULTS

Spider–Milliped Encounters. Of the 89 spiders that were offered millipeds, 83 attacked. Data from these attacks are summarized in Table 1. Sixty-nine millipeds survived the encounters. Of these, 33 were merely "inspected" by the spiders—that is, pounced upon and then released without ever being grasped in the spider's chelicers—under which condition they never emitted secretion. The other 36 survivors were grasped, but never persistently or strongly enough to be pierced by the chelicers. Twenty-eight of these did emit

Table 1. Course and outcome of spider attacks (*n* = 83) upon *G. marginata*

Course of attack	Millipeds that discharged secretion, no.	Condition of spiders (12 hr after attack)	
		Normal, no.	Sedated, no.
Milliped inspected, released unharmed (*n* = 33)	0	33	0
Milliped bitten, released unharmed (in <3 min) (*n* = 36)	28	34	2
Milliped lethally bitten, rejected uneaten or partially eaten (in 3.5–56 min) (*n* = 14)	13	3	11

secretion, often wetting the spider's mouthparts, which was sometimes the immediate prelude to their release. Two such spiders became sedated by the experience. The 14 millipeds that were killed died as a consequence of the bite; 13 gave off secretion and were held for protracted periods before the spiders eventually rejected them. Eleven of these spiders became sedated (Fig. 1 *C* and *D*).

Sedation was of slow onset and surprisingly long duration. None of the 13 spiders that became totally immobilized showed conspicuous symptoms within the hour after release of the milliped. However, 8 were already motionless within 4 hr, and all achieved that condition and proved unable to right themselves (our criterion for total sedation) within 12 hr. All eventually recovered completely. Five were normal after 24 hr, another 5 within 2–4 days, and the remaining 3 within 5–6 days. They eventually resumed feeding on mealworms and showed no noticeable long-range ill effects.

Secretory Output of Milliped. It is clear from the data (Table 2) that although there is a multiplefold increase in secretory output (both in volume and total quinazolinone content of secretion) with increase in milliped size, the values are remarkably constant when expressed as output per unit-body-mass. Therefore, the percent of body mass discharged as defensive quinazolinone is relatively constant, irrespective of body size ($\bar{x}$ ± SEM; 0.15% ± 0.02%). The molar concentration of the quinazolinones in the secretion is also relatively constant, as is the molar ratio of the two compounds.

These data provided the basis for selection of the quinazolinone dosage ranges used in the antifeedant and sedative potency tests.

Antifeedant Potency of Quinazolinones. The antifeedant doses (Table 3, first column) are high, certainly when viewed in relation to the quinazolinone output of *Glomeris*. For the glomerin/homoglomerin mixture the antifeedant dose corresponds to about 1/4th of the total quinazolinone discharged by a medium-sized (≈30 mg) milliped.

Homoglomerin was more potent as an antifeedant; the mixture of the compounds was of intermediate potency. Controls (not tabulated) were consistently inactive.

Many of the spiders in these tests became sedated, including individuals that had been tested with quinazolinone dosages well below antifeedant levels. This in itself pointed to a high systemic sensitivity of the spiders to the compounds.

Sedative Potency of the Quinazolinones: *per os.* As is evident from the data (Table 3, second column), the sedative doses with the three samples of synthetic quinazolinones were significantly lower than the corresponding antifeedant doses. The sample based on actual secretion, containing natural quinazolinones (bottom entry in column), was as effective (*P* > 0.7) as the closely corresponding synthetic mix-
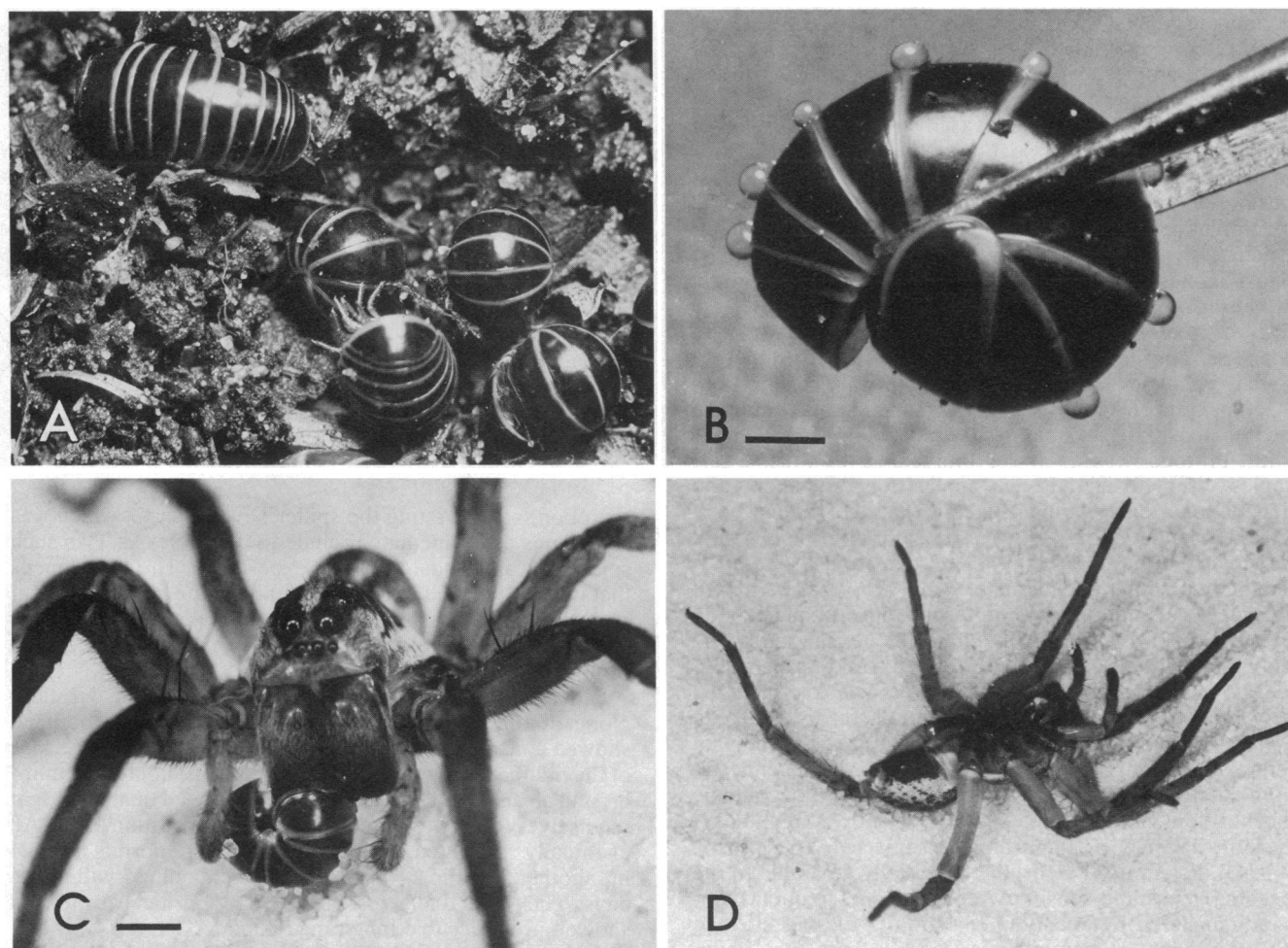
FIG. 1. (A) *G. marginata* on soil; specimens at right are seen in the typical coiled posture they assume when disturbed. (B) Coiled *Glomeris* responding to slight pinching with forceps by emitting secretion from the eight middorsal openings of its defensive glands. (Bar = 1 mm.) (C) *Lycosa* sp. in process of attacking a *Glomeris*, which is held, already injured, in the spider's chelicers. (Bar = 2 mm.) (D) Spider in state of sedation, hours after having attacked a *Glomeris*. The spider has here been flipped on its back with a glass rod and is failing to right itself, thus meeting the experimental criterion for sedation.

ture. This is evidence that the natural secretion derives its sedative potency exclusively from the quinazolinones.

Of the 270 spiders that became sedated in these tests, 229 recovered fully within 21 days and 2 died in that period; the remainder died in the subsequent 2–3 weeks without ever recovering mobility. None of the controls showed behavioral anomalies.

Sedative Potency of Quinazolinones: Injected. The data (Table 3, third column) confirm the high degree of systemic sensitivity of the spiders to quinazolinones. Sensitivity to the injected compounds was lower than to the ingested quinazolinones, which could be explained by the fact that injection was to the opisthosoma, at some distance from the major neural centers upon which the quinazolinones presumably act, while enteric absorption may have taken place closer to these centers.

A total of 324 spiders became sedated in these tests. Within 21 days after injection, 146 recovered fully and 109 died; a further 59 died in the ensuing 2–3 weeks. The combined group of 168 that died provided the basis for calculation of lethal doses (Table 3, last column). Control spiders showed no abnormalities.

DISCUSSION

The finding that a sedative drug used by humans has a close chemical counterpart in nature that effects its biological role

by inducing sedation is in itself interesting. Defensive chemicals produced by animals, including the very considerable number of substances now known from arthropods (10, 11), act largely by inducing immediate effects. By being distasteful, repellent, or irritating, they act as instantaneous antifeedants, without usually eliciting delayed effects. This is not to say that defensive chemicals never act by induction of toxic aftereffects. For instance, emesis induced by cardenolides ingested by birds with monarch butterflies is supposedly responsible for the learned aversion that birds can develop toward these insects (12). Plants, too, one would imagine, could derive benefit from poisons of delayed action, including emetics, antidigestive factors, hormone analogs, and, for that matter, sedatives (13, 14). One wonders, in fact, whether arborine, the quinazolinone from plants (15), might be adaptively justified in the source organism by its sedative action on herbivores.

The sedation elicited by *Glomeris* quinazolinones in *Lycosa* is noteworthy not only because of its long duration but because it is induced by levels of quinazolinones that are but a fraction of the total quantity of the compounds put forth by the millipeds with their secretion. The median sedative dose for the (equimolar) glomerin/homoglomerin mixture (0.7 μg per spider; *per os*) corresponds to about 1/85th of the total quinazolinone (≈60 μg) discharged by a medium-sized (≈30 mg) *Glomeris*. Thus, a single secretion droplet from such a *Glomeris* (= 1/8th total secretion), contains >10 times the

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Table 2. Volume and quinazolinone content of secretion of individual *G. marginata* ($n = 8$)

Milliped body mass, mg	Volume		G + H content		G + H concentration, M	G/H molar ratio
	μl per milliped	μl/mg of milliped	μg per milliped	μg/mg of milliped		
5	0.2	0.04	3	0.6	0.08	1.7
5	0.1	0.02	5	1.0	0.27	1.1
33	0.8	0.02	61	1.9	0.31	1.6
33	1.2	0.04	58	1.8	0.26	0.6
36	1.2	0.03	69	1.9	0.32	0.9
45	1.5	0.03	91	2.0	0.33	0.9
49	1.2	0.02	67	1.4	0.31	0.9
63	1.4	0.02	83	1.3	0.32	0.4
		0.028		1.49	0.28	1.01
		± 0.003		± 0.18	± 0.03	± 0.16

G, glomerin; H, homoglomerin. Averages below columns give $\bar{x} \pm$ SEM.

median sedative dose for a 350-mg spider. Mere contamination of the mouthparts with *Glomeris* secretion could thus entail considerable risk for a *Lycosa*. Indeed, in the staged encounters, two spiders became sedated after merely "mouthing" *Glomeris*.

Although the lethal dose of quinazolinones to *Lycosa* is relatively high (9.4 μg per spider for equimolar glomerin/homoglomerin, or about 1/8th of what is discharged by a medium-sized *Glomeris*), one can envision even sublethal ingested quinazolinone dosages being fatal to spiders, because it is unlikely that these could long survive exposure under natural conditions in a sedated state. They could fall victim to ants and other ground foragers or, unable to seek shelter as they ordinarily do in the daytime, die from desiccation.

Much has been written recently about aversive conditioning and about the ability of an animal to learn to associate a delayed ill effect with the dietary item that induced the effect. One wonders, therefore, whether spiders that survive sedation emerge the "wiser" from the experience and subsequently discriminate against *Glomeris*. Although we have not as yet tested for this eventuality, the notion that even invertebrates are capable of such associative learning has recently received experimental support (16, 17). It should be noted that *Glomeris* is protected against vertebrates also, having been reported to be rejected by anurans, birds, and mice. To mice, moreover, glomerin was shown to be systemically toxic (4).

Somewhat unexpected was the finding that the quinazolinones are less potent as antifeedants to *Lycosa* than as sedatives. The antifeedant doses are in fact at a par with the lethal doses for these compounds. This means that the spiders

lack an appropriately sensitive (peripheral or central) nervous gauging mechanism by which they could be "forewarned" against ingestion of poisonous levels of quinazolinones. The fact that so many of the spiders in our feeding tests with millipeds and quinazolinone-treated mealworms did become sedated is proof that they do indeed make the dietary "blunder" of ingesting too much quinazolinone. For the milliped the consequences can be fatal. Unable to use its secretion to effect quick release from a spider, it may fall victim (as frequently happened in the observed encounters) before sedation sets in to incapacitate the predator. The death of the milliped could, of course, still benefit its genetic kin, if these should share its habitat and be left to profit from the induced milliped aversion (or death following sedation) of the spider. It should be noted in this connection that our *Lycosa* stemmed from Florida, where *Glomeris* (and, to our knowledge, related millipeds) do not occur. Spiders that have evolved in sympatry with *Glomeris* might respond differently to the millipeds or fare differently from exposure to quinazolinones. Data that we will be presenting elsewhere on European lycosids show this to be so.

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Table 3. Sensitivity of spiders to quinazolinones

Compound	Antifeedant			Sedative *per os*			Sedative injected			Lethal injected	
	Dose, μg per spider	n	P	Dose, μg per spider	n	P	Dose, μg per spider	n	P	Dose, μg per spider	n
G	69.5 ± 24.4 (10–470)	4	<0.001	3.5 ± 0.5 (0.8–14)	6	<0.01	6.8 ± 0.7 (1.7–27)	6	<0.01	16.0 ± 2.3 (2.0–120)	6
H	7.5 ± 1.9 (0.9–64)	7	<0.001	1.4 ± 0.2 (0.2–9.8)	6	<0.001	4.1 ± 0.4 (1.1–16)	6	<0.01	19.2 ± 5.6 (1.5–240)	6
G + H (synthetic mixture)	15.4 ± 3.6 (1.8–140)	7	<0.001	0.7 ± 0.1 (0.1–4.6)	6	<0.001	3.2 ± 0.5 (0.6–17)	6	<0.001	9.4 ± 1.0 (2.6–33)	6
G + H (secretory origin)	—			0.7 ± 0.2 (0.03–16)	6		—			—	

G, glomerin; H, homoglomerin. Doses are probit medians (ED_{50}s) and are given as $\bar{x} \pm$ SEM, with calculated dynamic range in parentheses; n = number of doses used in probit analysis. Levels of significance between compared entries are indicated by P values.

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